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## Manuscript Details

<b>Manuscript number</b>	JEK_2019_56_R1
<b>Title</b>	SHEAR WAVE ELASTOGRAPHY INVESTIGATION OF MULTIFIDUS STIFFNESS IN INDIVIDUALS WITH LOW BACK PAIN
<b>Article type</b>	Research Paper

### Abstract

The purpose of this study was to investigate differences in passive muscular stiffness between the superficial multifidus (SM) and deep multifidus (DM), and to compare their passive and active stiffness in individuals with low back pain (LBP) and asymptomatic individuals. Fifteen LBP individuals and 15 asymptomatic individuals were recruited. Passive stiffness of the SM and DM was measured bilaterally using shear wave elastography (SWE) with participants lying prone. Active stiffness was measured for the SM during trunk extension, and the contraction ratio was calculated. DM displayed higher passive muscular stiffness than SM in both the asymptomatic and LBP groups ( $14.41 \pm 2.62$  and  $15.40 \pm 2.77$  kPa respectively;  $t=7.765$  and  $t=3.864$ ,  $p<0.05$ ). Individuals with LBP exhibited higher passive muscular stiffness of SM (LBP:  $10.15 \pm 4.21$ , asymptomatic:  $6.84 \pm 1.69$  kPa;  $t=3.002$ ,  $p<0.05$ ) and a lower contraction ratio (LBP:  $1.54 \pm 0.47$ , asymptomatic:  $2.65 \pm 1.36$  kPa;  $p<0.05$ ) compared to the asymptomatic group. The findings support a differentiation in passive muscular stiffness between SM and DM and provide evidence for an alteration in muscular stiffness at rest in individuals with LBP. The lower increase of muscular stiffness with contraction observed for those with LBP may reflect a deficit in activation of the multifidus.

<b>Keywords</b>	Low back pain; muscular stiffness, lumbar multifidus; shear wave elastography
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<b>Suggested reviewers</b>	Alessandro Schneebeli, Francois Hug

## Submission Files Included in this PDF

### File Name [File Type]

Cover Letter for resubmission.docx [Cover Letter]

Amendments table.docx [Response to Reviewers]

Abstract.docx [Abstract]

Manuscript.docx [Manuscript File]

Figure 1. SWE acquisition for SM and DM.pptx [Figure]

Figure 2. Passive and active muscular stiffness in asymptomatic.pptx [Figure]

Figure 3. Passive and active muscular stiffness in LBP.pptx [Figure]

Figure 4. Shear elastic modulus at rest for SM and DM.pptx [Figure]

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## **Research Data Related to this Submission**

There are no linked research data sets for this submission. The following reason is given:  
The data that has been used is confidential

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12<sup>th</sup> April 2019

Dear Editor,

Reference Manuscript: **Shear wave elastography investigation of multifidus stiffness in individuals with low back pain**

Thank you for your consideration and the reviewers' favourable comments on of the aforementioned manuscript. Please find enclosed our revised paper and detailed mapping of review comments and our revisions which we hope is agreeable to you and meet the threshold for publication in the Journal of Electromyography and Kinesiology.

The authors have contributed to the revisions of the manuscript. They have all agreed to submission of this manuscript to Journal of Electromyography and Kinesiology. The authors have no conflicts of interest to declare in the submission of this research for publication.

There have been no previous publications of this work.

I look forward to hearing your further consideration of the paper's suitability for Journal of Electromyography and Kinesiology.

Yours sincerely

A handwritten signature in dark ink, appearing to read 'Nicola Heneghan', written in a cursive style.

Nicola Heneghan  
PhD. MSc. MMACP.

<p><b>Ref: JEK_2019_56</b></p> <p>Title: SHEAR WAVE ELASTOGRAPHY INVESTIGATION OF MULTIFIDUS STIFFNESS IN INDIVIDUALS WITH LOW BACK PAIN</p> <p><b>Journal: Journal of Electromyography and Kinesiology</b></p>	
<p><b>Reviewer: 1</b></p> <p><b>Comments for the authors below:</b></p> <p>The purpose of this study was to investigate differences in passive muscular stiffness between the superficial multifidus (SM) and deep multifidus (DM), and to compare their passive and active stiffness in individuals with low back pain (LBP) and asymptomatic individuals. I think it is interesting biomechanical issue in subjects with musculoskeletal disorder such as LBP. The manuscript is well written and the design and methodology and statistical analysis is good.</p> <p>However, I think it is better to include the relationship between disability or fear of pain and the tested parameter.</p>	<p>Thank you for your very positive comments on this manuscript.</p> <p>We did not include the investigation of the relationship between disability or fear of pain with muscular stiffness because it is not within the scope of the current study. However, we agree with you about its importance (i.e. Do LBP patients with higher fear of movement/disability have higher stiffness?); and further research should address this research question.</p>
<p><b>Reviewer: 2</b></p> <p>The manuscript entitled “Shear wave elastography investigation of multifidus stiffness in individuals with low back pain” investigates the differences in passive muscular stiffness between the SM and DM, and the differences in passive and active muscular stiffness in individuals with LBP compared to asymptomatic individuals. The authors suggest that DM displayed higher passive muscular stiffness than SM in both the asymptomatic and LBP groups, and that LBP exhibited higher passive muscular stiffness of SM and a lower contraction ratio compared to the asymptomatic group. The experiment is well conducted, and the manuscript is clear and well written</p> <p><b>Comments for the authors below:</b></p>	<p>Thank you for your very positive comments on this manuscript.</p>
<p>First, to my knowledge the reliability of the technique used to measure the shear modulus has never been reported (i.e., LOGIQ S8 GE). Indeed, all the references (Moreau et al., 2016; Creze et al., 2017, and Koppenhaver et al., 2018) cited by the authors have used the Aixplorer device (Supersonic Imagine).</p>	<p>Thank you for highlighting this point. As you correctly pointed out, all cited studies used the Aixplorer (AIX) device (supersonic imaging) rather than LOGIQ to evaluate the reliability of the SWE measurements in the lumbar spine. Indeed, each system uses a different patent-protected technology that differs in terms of shear wave generation and tracking. However, a recent study revealed good agreement between AIX and LOGIQE9 in the quadriceps muscle [Alfuraih et al., 2017]. Also, a previous phantom study also reported that the performance of the LOGIQE9 was</p>

	comparable to the AIX [Song et al., 2015]. Since the technology behind the LOGIQE9 and the LOGIQ S8 device used in the current study is the same, we believe that reliability values observed using AIX for the measurement of the muscular stiffness of the lumbar region may be generalized to our device in some extent. However, we have also added our own reliability results (please see comment below).
Line 7 : "shear" Line 11 : "14.41±2.62" one decimal is precise enough Line 11 : please give the exact p values	Thank you very much for identifying this typing error in line 7; it has been amended. We would like to maintain two decimals for the mean and SD also in the abstract for consistency. We have amended p values.
Also If the authors have the data, I suggest to calculate active shear modulus as follows : active shear modulus = shear modulus measured during active contraction – passive shear modulus (Avrillon et al., 2018 JEK; Raiteri et al., 2016 J Biomech).	Thank you very much for your feedback. Two different methods for the calculation of the contraction ratio has been reported in the literature. $\frac{\text{active muscular stiffness}}{\text{passive muscular stiffness}} = \text{contraction ratio}$ (reported by Botanlioglu et al. [2013]) And  active muscular stiffness – passive muscular stiffness= contraction ratio. (reported by Avrillon et al. [2018])  We thank you for your recommendation, which is also a very appropriate method to calculate the shear elastic modulus with contraction. We would like, however, to maintain the approach used by Botanlioglu et al. [2013]. We feel that this approach is the best for our study since we also compare our findings with those reported by Dieterich et al. [2017]; who normalized the muscular stiffness with contraction dividing by the stiffness values at rest.
Line 16 : "DM and provide evidence for an alteration in muscular stiffness at rest in individuals with LBP". Please, check the reliability and saturation of the elasticity maps.	Thank you very much for raising this issue. The reliability values have been included in the manuscript (lines 123-124; 147-149). As two acquisitions were taken per each muscular stiffness measurement (passive muscular stiffness SM and DM and active muscular stiffness for SM), the intra-rater reliability of the SWE acquisitions (mean of 9 elastograms) was examined using two-way mixed-effects model [ICC (3.1)]. Data was taken from the right side of the asymptomatic participants.  The results, which you can find now in the manuscript, are: The ICC values (95% confidence interval) were 0.92

	<p>(0.79-0.97) and 0.90 (0.72-0.97) for shear elastic modulus at rest of the SM and DM respectively (which are considered excellent); and 0.81 (0.51-0.94) for shear elastic modulus of the SM with contraction.</p> <p>The wider range observed for the reliability of muscular stiffness with contraction may reflect that participants may have used slightly different strategies for the trunk extension between the two active muscular stiffness acquisitions.</p> <p>On the other hand, saturation of the elasticity/quality maps were not created in the current study. We used a similar procedure as MacDonald et al. [2016], which consists of eliminating elastograms with artefacts such as 'holes' caused by an attenuation effect avoid under- or over-estimation of muscular stiffness values. However, it must be pointed out that this was the case of very few elastograms.</p> <p>When this study was conducted, contemporaneous SWE studies lacked of the evaluation of the quality maps before SWE acquisition and so, they were not included. Therefore, we recognized this limitation of our study.</p>
<p>To my view the active condition is very difficult to interpret. Neither the intensity nor the lombar angle are described is the method section while both influence the shear modulus.</p> <p>Line 17 : "The lower increase of muscular stiffness with contraction observed for those with LBP may reflect a deficit in activation of the multifidus». This may be related to the intensity of the submaximal task.</p>	<p>Thank you for your feedback. In the present study, the angle of the trunk extension was visually monitored, as it was originally described by Ito et al. (1996), rather than standardized with a device (i.e. an electrogoniometer). The angle of trunk extension was ~15° according to Ito et al. (1996)</p> <p>We agree that there may have been subtle differences in range of motion which could not be detected visually. However, even if exactly the same range of motion was performed, it is expected that the LBP subjects may have activated their muscles differently and use different strategies [Hodges and Tucker, 2011].</p>
<p>Line 34 : "This study stands to provide novel insights into the normal mechanical properties of the multifidus muscle and how this is modified in individuals with LBP". Did you measure EMG activity during passive measurements to ensure that the LBP are fully relaxed.</p> <p>Does the pain of LBP patients induce a slight contraction which can be detected by elastography ?</p>	<p>Thank you very much for your feedback. EMG activity was not measured during the passive measurements. Following recommendations, we allow participants for 5 min lying down (relaxed) before starting the measurement of the passive muscular stiffness to guarantee a resting phase of the muscle [Kot et al., 2012; Alfuraih et al., 2018]. This procedure has been previously followed in the examination of the muscular stiffness of the lumbar muscles too [Creze et al., 2017]. This information can be found in lines 84-86.</p> <p>Evidence is conflicting about whether or not people with LBP exhibit an increased muscular</p>

	<p>activity in resting postures [Geisser et al., 2005]. To investigate if a greater passive muscular stiffness of the lumbar muscles is related to an increase of muscular activity at resting (i.e. in prone) was not within the scope of this study; but it may provide new insights into the muscular impairment in people with LBP and should be explored in future research.</p>
<p>Line 67 Participants were positioned in prone with a rolled towel positioned under their abdomen to minimize the lumbar lordosis [Stokes et al., 2007]. Did you measure the lumbar lordosis for all the participants ? Does the towel was positioned by the examiner or the subjects ?</p>	<p>Thank you for raising this issue. Participants were positioned in the plinth according to recommendations [Stokes et al., 2007]. This position has been used in previous SWE studies investigating muscular stiffness of the lumbar muscles [Moreau et al., 2016]. The lumbar lordosis was not measured, and it may be a source of variability across participants. Masaki et al. [2017], who found greater muscular stiffness for multifidus in LBP individuals when compared to asymptomatic, did not find between group differences for lumbar lordosis.</p> <p>The towel was positioned by the examiner, who also checked afterwards to guarantee that it was placed in the right position before starting the measurement.</p>
<p>Line 89 : “nine continuous elastograms for SM and DM”. It is not very clear to me, can you provide more details.</p>	<p>The mean of 2 acquisitions was calculated to obtain the shear elastic modulus values for muscular stiffness at rest (DM and SM) and with contraction (SM). For each acquisition, the probe was maintained motionless on the skin until 9 elastograms were recorded. The software of the LOGIQ S8 allows for the recording of continuous elastograms in a “movie mode”. After the acquisition, the 9 elastograms are individually inspected to evaluate if artefacts are present and then the mean is calculated.</p>
<p>Line 90 : “(~15°)” of what ?</p> <p>Line 90 : “rest between repetitions” What was the submaximal task ?</p>	<p>Thank you very much for your feedback. Further information has been added for clarification in lines 90-92.</p> <p>Active muscular stiffness was evaluated during trunk extension in prone position (submaximal task). Two repetitions were requested and a 10-second rest between repetitions was allowed.</p>
<p>Line 103 : Please use shear elastic modulus instead of muscular stiffness throughout the manuscript.</p>	<p>Thank you very much for your feedback. The term “muscular stiffness” has been replaced with “shear elastic modulus”. We have, however, sometimes retained the term “muscular stiffness” since we</p>



	believed it was more appropriate due to the specific context.
Line 123 : Can you precise the post hoc analysis used please ?  Line 143 : Can you provide to the readers the exact p values please ?	Thank you very much for your feedback. Sections “statistical analysis” (lines 110-124) and “results” (lines 134-149) have been amended to provide further information following reviewers’ suggestions.
<p><b>Reviewer: 3</b></p> <p>General comments</p> <p>Thank you for inviting me to review this manuscript. This study compared passive muscular stiffness: (1) between the superficial multifidus (SM) and deep multifidus (DM); (2) between individuals with LBP and asymptomatic individuals. A total of 30 participants (15 each group) took part in the study. Findings suggest that: (1) DM has higher passive muscular stiffness than SM in the asymptomatic and individuals with LBP; (2) individuals with LBP have higher passive muscular stiffness of SM and a lower contraction ratio compared to the asymptomatic group.</p> <p>This is an interesting study, but some revision is required to enhance its clarity.</p>	Thank you for your very positive comments on this manuscript.
<p>Specific comments</p> <p><b>Abstract:</b></p> <p>“...findings support a differentiation...”: minor comment. Avoid nominalization (i.e. “differentiation”). That impacts on readability of your manuscript.</p>	Thank you very much for raising this issue; it has been amended through.
<p><b>Introduction:</b></p> <p>You did not mention about contraction ratio in your aims. I am unclear which “aim” of the study is the contraction ration analysis and comparison linked to.</p>	<p>Thank you very much raising this point. We could not explain and describe the term contraction ratio in the introduction due to word count. For that reason, we decided to not include it in the description of the objectives of the study.</p> <p>However, we have now amended and change passive and active muscular stiffness with muscular stiffness at rest and with contraction (lines 28-30). We think that it is clearer, and in this way, we introduce and describe the contraction ratio as measure of muscular stiffness with contraction in the methods section.</p>
Pages 1 and 2, lines 16 to 27: seems that you have two different ideas being presented in this paragraph. The topic sentence suggests the focus is on SWE and its psychometric properties.	Thank you very much for your feedback and we totally agree with your suggestion of further discussion may be an added value. However, due to the word count, we decided to include the

I think you should a new paragraph and discuss and more in-depth the conflicting findings from the two studies that have assessed passive muscular stiffness on multifidus. In its current version, this is too briefly presented to the reader.	discussion of findings from previous research only in the discussion.
<b>Methods:</b> Did you estimate your sample size <i>a priori</i> ? This needs to be clarified.	Thank you very much for raising this issue; this is an important point. As there is not available data from previous studies for the comparison between SM and DM and previous research has compared the entire multifidus muscle between asymptomatic and LBP individuals, sample size was not able to be calculated a priori. The comparison for DM between LBP and symptomatic did not achieve enough statistical power ( $1-\beta < 0,8$ ), and a bigger sample should be needed. However, as this was the only comparison without statistical power and a large effect size was observed for the rest of comparisons ( $d > 0.08$ ), we are wondering about the meaningfulness of the differences in muscular stiffness of the DM between groups if detected. The present study provides data which will allow future research to calculate sample size a priori.
Line 65, “using”: re-word sentence to avoid repetition and improve flow?	Amended.
Line 90, “, (~15o).”: apologies, but that is unclear.	Thank you very much. Further clarification on this issue has been also requested for another reviewer and it is now amended on the text.
Line 91: what did the examiner monitor? What criteria was used to consider participants performed the task successfully?	<p>Examiner visually monitored that participants hold the correct position (~15°) without dropping.</p> <p>During the Ito test, participants has to hold the position for 5 min or up to exertion. However, in the current study, participants were only required to maintain the trunk extension until 9 elastograms were recorded (around 15 sec). Therefore, none of the participants (pain-free or LBP) showed exertion of the task (drop the trunk extension position).</p>
Lines 96 to 97, “Previous studies have reported poor quality signal during the evaluation of the deep abdominal muscles during contractions [MacDonald et al., 2016].”: this sentence seems out of context. It reads more like a discussion.	We agree with your opinion and we have changed this sentence to the section “methodological considerations”.
Line 112, “... (absolute values).”: minor comment. I think you should explicitly state that you used absolute values (e.g. This was done using absolute values). Its current format seems like “text messaging”.	Thank you very much for your feedback. We understand your concerns about this point, but we would like to maintain it unchanged. As absolute values of muscular stiffness with contraction are only used to calculate the contraction ratio and not in the statistical analysis, we believe that the original sentence is the clearest for the reader.

<p>Lines 121 to 126: this section could be revised to improve clarity. I am not clear what exactly was done in terms of statistical analysis. Did you use the two-way repeated measures analysis of variance (ANOVA) for comparing passive muscular stiffness: (1) between the superficial multifidus (SM) and deep multifidus (DM); (2) between individuals with LBP and asymptomatic individuals? Did you use planned contrasts? Please justify your approach. Did you adjust alpha for multiple comparisons? Please justify your approach.</p> <p>Please be explicit about which variables you are using the dependent variables and independent variables. I suggest you being consistent with terms used before, so that it is clear for the reader which variables you are referring to. Did you use independent t-test as the post hoc analysis? It seems so, but the way it is written, it seems that your post hoc analysis is one thing, and your independent t-test is something else</p>	<p>Thank you very much for pointing out this issue and we believe that further clarification is needed on the statistical test.</p> <p>As you currently pointed out, we used two-way repeated measures analysis of variance (ANOVA) for comparing <b>passive muscular stiffness</b>: (1) between the superficial multifidus (SM) and deep multifidus (DM); (2) between individuals with LBP and asymptomatic individuals. The multiple comparison was planned a priori (Pairwise comparisons with Bonferroni)</p> <p>Also, as muscular stiffness was not able to be measured with contraction for the DM (due to signal noise), independent samples t-test were used to compare this variable between LBP and asymptomatic participants.</p> <p>The sections statistical analysis and results have been amended for further clarity.</p>
<p>This is the first time you use the term “muscle layer”. As a reader, I suspect what you are referring to, but that requires me to make assumptions.</p>	<p>Thank you very much for noticing this issue; the term “layers” has been replaced with “fibers”.</p>
<p>Lines 136 to 148: this section could be revised and have a more clear structure, with findings for each “comparison” being presented in a systematic way. The discussion section has better structure.</p>	<p>Thank you very much for your feedback and tips for guarantee an appropriate development of the results section.</p> <p>The entire results section has been amended for further clarity following your suggestions.</p>
<p>You set alpha at 0.05, not “p” (which is an outcome, i.e. the probability, of your statistical analysis).</p>	<p>Thank you very much for your feedback, this has been amended.</p>
<p><b>Results</b></p> <p>Please also report the degrees of freedom for your “t” test. I think you would add strength to your findings if you report the 95% CI for the difference between the groups (LBP vs asymptomatic) and muscle layers (DM and SM). When reporting comparisons, I suggest you following a structured approach (e.g. <a href="https://statistics.laerd.com/spss-tutorials/independent-t-test-using-spss-statistics.php">https://statistics.laerd.com/spss-tutorials/independent-t-test-using-spss-statistics.php</a>).</p>	<p>Thank you very much for your feedback on this issue. 95% CIs are now reported in table 2.</p>
<p>Lines 141 to 144: no reference is made to the table presenting the mean values for the different groups.</p>	<p>Thank you very much for raising this issue. Reference to the table has been added in line 141.</p>
<p><b>Discussion</b></p> <p>“Functional differentiation between the DM and SM”: the term “differentiation” impacts on readability. You could simplify this and state “functional differences between the DM and SM”.</p>	<p>These terms have been amended accordingly.</p>
<p>Line 170 to 173, “It has been...”: long sentence. Hard to follow. Could be revised to improve clarity.</p>	<p>Thank you very much for your feedback. This paragraph has been modified for further clarification.</p>

Lines 168 to 182: this paragraph could be revised for clarity. The topic sentence does not reflect what is discussed in the paragraph (i.e. type of muscle fibres).	The aim of this paragraph is to discuss our findings together with previous histological research on the differences between SM and DM.
Line 183, "Changes in multifidus stiffness in individuals with LBP": this is not accurate. You did not assess "changes" in that group, but you assessed differences in multifidus stiffness between individuals with LBP and asymptomatic participants. Do assess "changes", you would need repeated measures with the same participants over time. The "subheading" should reflect that.	Thank you for highlighting this point and yes, we agree. This has been amended accordingly.
Lines 195 to 198: very long sentence. Hard to follow. Please revise and use a clear topic sentence.  Lines 195 to 213: this paragraph needs revision to improve its clarity. I found it hard to follow what the topic of the paragraph is.	Thank you very much for indicating the lack of clarity of this paragraph. The aim is to present previous research on muscle structure/morphology in people with LBP to explain the current findings and those reported by Masaki et al. [2017] (1), as well as to explain the disagreement with the findings reported by Chan et al [2012] (2).  The structure of the paragraph has been modified to achieve these two goals and gain clarity.
Lines 227 to 242: what is the "topic" of this paragraph? You need a clear topic sentence to flag to the reader what will be discussed in this paragraph.	Thank you very much for your feedback. however, we would like to maintain this paragraph unchanged.  The aim of this paragraph was to compare our findings with previous EMG evidence. We believe that this comparison is possible in some degree since previous research has shown a positive linear relationship between muscular stiffness, contraction and the level of muscular activity and muscle force [Nordez and Hug, 2010; Yoshitake et al., 2014; Ateş et al., 2015].  Consequently, we aimed to suggest how our findings may provide some insights into the relationship between muscle structure and function of the multifidus muscle in people with LBP. However, further research in this vein is needed.
Lines 241 to 242, "Therefore, the current results suggest that SWE could be used to identify contractile deficits of the multifidus in individuals with LBP, however this remains speculative.": you are contradicting yourself in this statement. After all, can you use SWE or not? I would argue that your study did not assess that and therefore, you cannot make this claim.	Thank you for highlighting this point and we agree with your opinion. Thus, this sentence has been eliminated.
Tables Captions should be presented before the table...	Amended.
Figures 4 and 5 Please add to caption what the error bars mean	Included at the end of the reference list along with titles for each figure



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1

## 2 **Abstract**

3 The purpose of this study was to investigate differences in passive muscular  
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7 individuals were recruited. Passive stiffness of the SM and DM was measured  
8 bilaterally using shear wave elastography (SWE) with participants lying prone. Active  
9 stiffness was measured for the SM during trunk extension, and the contraction ratio  
10 was calculated. DM displayed higher passive muscular stiffness than SM in both the  
11 asymptomatic and LBP groups ( $14.41 \pm 2.62$  and  $15.40 \pm 2.77$  kPa respectively;  
12  $t=7.765$  and  $t=3.864$ ,  $p<0.05$ ). Individuals with LBP exhibited higher passive  
13 muscular stiffness of SM (LBP:  $10.15 \pm 4.21$ , asymptomatic:  $6.84 \pm 1.69$  kPa;  $t=3.002$ ,  
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19 in activation of the multifidus.

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3 **SHEAR WAVE ELASTOGRAPHY INVESTIGATION OF MULTIFIDUS STIFFNESS**  
4 **IN INDIVIDUALS WITH LOW BACK PAIN**  
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7 Rushton (PhD), Nicola R Heneghan (PhD)  
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12 Environmental Sciences, University of Birmingham, UK  
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15 This study was approved by the University of Birmingham ethics committee and the  
16 procedures were conducted in agreement with the Declaration of Helsinki (ERN\_17-  
17 0782).  
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## **Introduction**

Multifidus muscle has brought the attention of electromyographic (EMG) research in recent years, being identified as key muscle in the rehabilitation of people with low back pain (LBP) [MacDonald et al., 2006]. EMG research has supported first, a functional differentiation between the superficial (SM) and deep fibers of the multifidus (DM) and second, impaired function of this muscle in people with LBP [Danneels et al., 2002, Moseley et al., 2002, MacDonald et al., 2009]. It has been theorized that both, the functional differentiation between multifidus fibers and the functional impairment observed in people with LBP, may be related to the muscle structure, but research in this vein is inconclusive [Porterfield and DeRosa, 1998, Cagnie et al., 2015]. However, investigating the mechanical properties of muscle, such as muscular stiffness, may offer a better understanding of variation within the multifidus fibers and the relationship between muscle structure and normal/altered function [Brandenburg et al., 2014, Roberts, 2016].

Shear wave elastography (SWE) provides a non-invasive quantitative measure of muscular stiffness at rest and during a contraction, which has shown to be positively related to the level of muscular activity and muscle force [Nordez and Hug, 2010, Brandenburg et al., 2014, Yoshitake et al., 2014, Ateş et al., 2015]. SWE has previously been used to investigate the stiffness of the lumbar multifidus of asymptomatic individuals at rest and during contraction with good to excellent reliability (intra class correlation coefficients (ICC) values of between 0.77 to 0.94) [Moreau et al., 2016, Creze et al., 2017, Koppenhaver et al., 2018]. However, no study has investigated whether or not differences in muscular stiffness exist between the SM and DM. Furthermore, only two studies have investigated passive muscular

86 stiffness of multifidus in people with LBP, but the results are conflicting [Chan et al.,  
87 2012, Masaki et al., 2017].

88         In this study, we investigate (1) whether differences in passive muscular  
89 stiffness exist between the SM and DM in asymptomatic and LBP individuals and (2)  
90 if differences in passive and active muscular stiffness exist in individuals with LBP  
91 compared to asymptomatic individuals. This study stands to provide novel insights  
92 into the normal mechanical properties of the multifidus muscle and how this is  
93 modified in individuals with LBP.

## **Methods**

### **Participants**

Fifteen individuals with LBP and 15 asymptomatic were recruited from staff and student communities at the University of Birmingham. All participants were eligible for this study if they were aged between 20-55 years, with 55 chosen as the maximum age to reduce the effect of age-related adipose infiltration within the muscle [Marcus et al., 2010]. The LBP group included participants who had reported continuous LBP for more than 3 months or non-continuous pain for greater than 6 months with pain on at least half of the days [Krismer and Van Tulder, 2007]. The asymptomatic group included participants without history of LBP. Exclusion criteria for both groups included neurological or respiratory disorders, pregnancy or previous spinal surgery. Individuals with LBP must not have been receiving treatment from a health care professional at the time of recruitment. Additional exclusion criteria for the LBP group included no known underlying pathology such as spinal stenosis, vertebral fracture, disc herniation, radicular low back pain with neurological deficit suggesting nerve root compression and/or ankylosing spondylitis [Krismer and Van Tulder, 2007]. Ethical approval was granted by the University of Birmingham ethics committee (ERN\_17-0782) and the procedures were conducted in agreement with the Declaration of Helsinki. Informed written consent was obtained from all participants.

### **Questionnaires**

Participants with LBP completed the Numerical Rating Scale (NRS) to assess their pain intensity on the day of the measurement session and were also asked to rate their usual level of pain during the previous week. Additionally, the Oswestry

Disability Index (ODI) and Tampa Scale for Kinesiophobia (TSK) were used to assess perceived disability and fear-avoidance behavior respectively [Vlaeyen et al., 1995, Fairbank and Pynsent, 2000].

## **Procedure**

Stiffness of the SM and DM was measured bilaterally using an ultrasound imaging device with SWE (LOGIQ S8 GE Healthcare, Chicago USA) using a 9-linear array probe. All measurements were performed by the same experienced examiner trained in SWE measures. Participants were positioned in prone with a rolled towel positioned under their abdomen to minimize the lumbar lordosis [Stokes et al., 2007]. The ultrasound probe was placed 2cm lateral to the level of the third lumbar spinous process (L3), which corresponds with the space between transverse process of L3 and L4; confirmed by the ultrasound image. The probe was placed on the skin with minimal pressure across all participants [Cortez et al., 2016]. As muscle tissue is anisotropic, the ultrasound B-mode was used to identify the parallel orientation to the muscle fibers of SM; so the probe was positioned rotated towards the midline approximately 10° and also tilted approximately 10° from the sagittal plane [Cortez et al., 2016]. Once the orientation of the muscle fibers was identified, the outline of the probe was marked on the participant's skin to ensure consistency in placement across measures. For the DM, it was not possible to identify the orientation of the fibers. The multifidus muscle was divided in two equal region of interest (ROI), which were located under the thoracolumbar fascia (TLF) (without including it) for the SM, and just below this position and above the articular processes of the vertebrae for the DM (figure 1). As the ROIs were defined to include the larger SM and DM area possible, these were different across participants.

To measure passive muscular stiffness of the SM and DM, the SWE acquisition commenced after five minutes of lying to ensure that the muscle was at rest [Creze et al., 2017]. The probe was placed on the area marked previously and was kept motionless for five seconds to obtain a well-defined elastography frame [Koo et al., 2013]. Then, two acquisitions on each side allowed recording of nine continuous elastograms for SM and DM. Active muscular stiffness measures of the SM were acquired during an isometric trunk extension akin to Ito test [1996], ( $\sim 15^\circ$ ). The examiner monitored the participants performance visually [Ito et al., 1996]. The SWE acquisition commenced when the participant reached the trunk extension position, and nine elastograms were acquired twice on each side with a 10-second rest between repetitions. Active muscular stiffness of the DM was not included in the present study due to the poor-quality signal observed during the pilot sessions. Previous studies have reported poor quality signal during the evaluation of the deep abdominal muscles during contractions [MacDonald et al., 2016].

### **Image processing**

After the SWE acquisition, an area was circled over the ROI for all saved elastograms. Elastograms with artefacts such as 'holes' caused by an attenuation effect were eliminated to avoid under- or over-estimation of muscular stiffness values [MacDonald et al., 2016]. The muscular stiffness ( $\mu$ ) within each ROI was automatically calculated by the SWE software. Muscular stiffness measured in shear elastic modulus was obtained according to the formula  $\mu = \rho v^2$ , where  $\rho$  is the density of the muscle tissue (assumed to be  $1000 \text{ kg/m}^3$ ) and  $v$  is the shear wave propagation velocity [Gennisson et al., 2013]. The mean of the two acquisitions was calculated to obtain representative values for each muscular stiffness measure [Masaki et al., 2017]. To quantify the increase of muscular stiffness with contraction,

the contraction ratio [Botanlioglu et al., 2013] was calculated for the SM by dividing the mean passive muscular stiffness from the mean active muscular stiffness (absolute values).

## **Statistical analysis**

Descriptive statistics were used to analyze demographic data with inferential analysis including parametric and non-parametric tests used to compare groups. The Shapiro-Wilk normality test did not reveal significant deviation from normality for the measures of passive muscular stiffness and contraction ratio and paired-samples t-tests revealed no differences between sides for all muscular stiffness measures, so the mean of the right and the left side was calculated for further analysis.

A two-way repeated measures analysis of variance (ANOVA) (with group as the between-subject independent variable and muscle layer as within-subject factor) was performed to investigate if differences in passive muscular stiffness of the SM and DM existed within and between groups. Post hoc analysis was used to evaluate significant differences. Independent samples t-tests were performed to compare the contraction ratio of the SM between groups. Significance was set at  $p < 0.05$ .

## **Results**

### **Population Characteristics**

The characteristics of both groups are presented in Table 1. Both groups were comparable in age, gender, and BMI, with no significant differences seen between groups. The LBP group showed low disability and pain, with an average reported pain level at the time of data collection of  $2.27 \pm 1.62$  out of 10.

### **Muscular Stiffness**

Figures 2 and 3 show representative elastograms to determine passive muscular stiffness of the SM and DM, and active muscular stiffness of the SM for an asymptomatic individual and a person with LBP. There was a significant difference between the passive stiffness of the SM and DM as determined by the repeated measures ANOVA ( $F = 67.7$ ,  $p < 0.001$ ). Post hoc comparisons revealed that passive muscular stiffness was higher in the DM than the SM in both groups ( $p < 0.05$ , LBP:  $t = 3.864$ , asymptomatic:  $t = 7.765$ ). Moreover, passive muscular stiffness of the SM was greater for the LBP group relative to the asymptomatic group ( $p < 0.05$ ,  $t = 3.002$ ). However, no significant differences were found between groups for the passive stiffness of the DM ( $p > 0.05$ ,  $t = 0.898$ ) (Table 2, Figure 4). An independent samples t-test revealed a lower contraction ratio for the LBP group compared to the asymptomatic controls ( $1.54 \pm 0.47$  and  $2.65 \pm 1.36$ ,  $p < 0.05$ ) (Figure 5).



## **Discussion**

This is the first study to investigate whether differences in passive muscular stiffness exist between the DM and SM both in asymptomatic participants and in people with LBP. The findings illustrate a difference in muscular stiffness between the SM and DM, supporting the existence of a differentiation between the deep and superficial layers of the multifidus [MacDonald et al., 2009, Moseley et al., 2002]. In addition, individuals with LBP exhibited increased muscular stiffness of the SM at rest, and a reduced ability to stiffen this muscle with isometric trunk extension compared to asymptomatic individuals.

### **Passive muscular stiffness of SM and DM**

Passive muscular stiffness differed between the layers of the multifidus, with the DM displaying greater stiffness. Previous studies have evaluated stiffness of the multifidus but without differentiation between the DM and the SM or they have only examined the SM [Chan et al., 2012, Moreau et al., 2016, Masaki et al., 2017]. In line with the current findings, higher passive muscular stiffness has been observed for the deep posterior cervical muscles relative to the superficial muscles using SWE [Dieterich et al., 2017].

The greater stiffness found for the DM supports the hypothesis that the deeper fibers play a functional role in providing spinal support [MacDonald et al., 2006]. It has been hypothesised that functional differentiation between the DM and SM may be reflected in a higher proportion of type I fibers in the DM; which are more fatigue resistant than type II and so, ideally suited to hold low load tonic activity contributing to the postural control [Porterfield and DeRosa, 1998]. Although histological research is inconclusive due to sample bias, functional MRI have

revealed differences in the relaxation time between SM and DM, suggesting that the DM has a higher percentage of type I fibers compared to the SM [Dickx et al., 2010, Cagnie et al., 2015]. In vitro animal studies have confirmed that type I fibers are stiffer than type II [Goubel and Marini, 1987, Petit et al., 1990]; and therefore, together with previous research, the current findings lend support to the existence of a structural differentiation between the SM and DM which may have a functional implication.

### **Changes in multifidus stiffness in individuals with LBP**

Higher passive muscular stiffness of the SM was found for the LBP group when compared to asymptomatic participants. Masaki et al [2017] previously reported significantly higher passive muscular stiffness of multifidus (measured at the level of L4) in individuals with LBP, however, Chan et al [2012] did not observe group differences even if multifidus was examined at the same spinal level. In both studies, the ROI covered both the SM and DM and therefore, any potential differences between groups for SM muscular stiffness may have been concealed by the DM values. Furthermore, Chan et al [2012] utilized strain elastography, which is more operator dependent, potentially influencing their results [Brandenburg et al., 2014].

Passive stiffness is not only attributed to the contractile tissue within the muscle, and the increase of connective tissue due to a fibrotic proliferation may increase the shear elastic modulus values, explaining the current findings and those reported by Masaki et al [2017] [Gillies and Lieber, 2011, Brown et al., 2018]. Interestingly, Brown et al [2011] induced lumbar disc degeneration in rabbits and

found that, though the individual paravertebral muscle fibers became stiffer, the fiber bundles (composed of both muscle fibers and connective tissue) displayed a greater increase in stiffness. By contrast, the findings reported by Chan et al [2012] may be explained because of the higher adipose tissue infiltration found in the LBP group, which may have decreased the muscular stiffness and concealed the between group differences [Roskopf et al., 2015]. It has been found that the fat infiltration within multifidus may be caused by aging rather than by presence of pain [Lee et al., 2017]. This may explain the higher adipose tissue infiltration reported by Chan et al [2012] in the LBP group, which was older than the control group. On the other hand, the current findings of higher muscular stiffness may be due to a low level of adipose tissue infiltration in our LBP group, which was relatively young. In addition, though all participants had LBP for longer than 6 months, nearly all of them had non-continuous LBP and, therefore, may also exhibit a low amount of adipose tissue infiltration [Goubert et al., 2017].

#### **Differences in Contraction Ratio**

The participants with LBP presented a significantly lower contraction ratio; reflective of a smaller increase of muscular stiffness with contraction. The contraction ratio has previously been used to compare the increase of muscular stiffness with contraction between different conditions (pain/no pain) or between different muscles/muscle layers [Botanlioglu et al., 2013, Dieterich et al., 2017]. As a normalized measurement for each participant, where muscular stiffness at rest differs between conditions, the contraction ratio allows for a more accurate estimation of differences in stiffness with contraction and the force generation [Botanlioglu et al., 2013, Dieterich et al., 2017]. Similar to the current findings, lower

normalized active muscular stiffness was found in the deeper posterior neck muscles during isometric neck extension in individuals with neck pain [Dieterich et al., 2018].

As previous research has shown a positive linear relationship between muscular stiffness, contraction and the level of muscular activity and muscle force, the current results may be compared in some extent to findings from EMG studies that investigated the activation of the SM during isometric contractions [Nordez and Hug, 2010, Yoshitake et al., 2014, Ateş et al., 2015]. In agreement with the current findings, reduced activation of the multifidus has been observed during trunk extension in a prone position in individuals with acute and experimental LBP [Danneels et al., 2002, Dickx et al., 2008]. It is speculated that this deficit in contraction found in individuals with LBP (reflected by a lower increase of muscular stiffness), may be explained in part by the proliferation of collagen content/connective tissue hypothesized above based on the finding of higher muscular stiffness at rest. These changes within the muscle would result in a decrease in the amount of contractile tissue and subsequently reduced ability to perform an efficient contraction [Goubert et al., 2017]. Therefore, the current results suggest that SWE could be used to identify contractile deficits of the multifidus in individuals with LBP, however this remains speculative.

### **Methodological Considerations**

A limitation of SWE is the large inter-individual variability. Given that the SWE acquisitions were performed at a specific vertebral level and at a standardized distance from the spinous process, intra-muscular variations and regional differences likely explain a small extent of the variability with in the current data [Cortez et al., 2016, Stokes et al., 2007]. The higher variability in passive muscular stiffness of the

SM in the LBP group likely reflects the large variability of individual neuromuscular adaptations due to LBP and/or an increase of the amount of non-contractile tissue [Hodges et al., 2013, Brown et al., 2018]. Although elastograms with artefacts were removed from the analysis, the attenuation effect of the ultrasound push beam can be greater in the deep lumbar region due to the TLF, and might have generated artificial areas of very low/high stiffness, altering the muscular stiffness measurement and concealing the detection of significant differences between groups for the DM [MacDonald et al., 2016]. Also, as trunk position was controlled visually as Ito et al [1996] originally described, we cannot exclude small differences in trunk angle between groups, which could have affected measurements for active stiffness. Additionally, as LBP participants were not under treatment, the levels of pain and disability were fairly low; and so, different results may be obtained for individuals with more severe symptoms.

In conclusion, the present study provides new insights into the mechanical properties of the lumbar muscles. Specifically, the study demonstrates a difference in muscular stiffness between the DM and SM, with a higher muscular stiffness observed for the DM in both asymptomatic and LBP individuals. Greater passive muscular stiffness in the SM was found in individuals with LBP. Finally, a deficit in the contraction of the SM during an isometric trunk extension task was observed for those with LBP, reflected by a lower increase of muscular stiffness with contraction.

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**Figure 1.** Representative elastograms recorded from an asymptomatic *participant* for the SM (A) and DM (B). The layers of tissue are marked from superficial to deep; Subcutaneous tissue (1), TLF and erector spinae aponeurosis (2), multifidus muscle (3) and transverse process of L4 (4). The white dashed line represents the junction between the multifidus and the vertebral processes. Blue colours signify lower muscular stiffness values measured in shear wave velocity (m/s) and red colours signify higher muscular stiffness values.

**Figure 2.** Representative elastograms recorded from an asymptomatic participant; passive muscular stiffness of the SM (A), passive muscular stiffness of the DM (B) and active muscular stiffness of the SM (C).

**Figure 3.** Representative elastograms recorded from an LBP participant; passive muscular stiffness of the SM (A), passive muscular stiffness of the DM (B) and active muscular stiffness of the SM (C).

**Figure 4.** Shear elastic modulus at rest (passive muscular stiffness) of the Superficial Multifidus (SM) and Deep Multifidus (DM) for LBP and asymptomatic groups. \* $P < 0.05$

**Figure 5.** Contraction ratio of the superficial multifidus (SM) for LBP and asymptomatic groups.

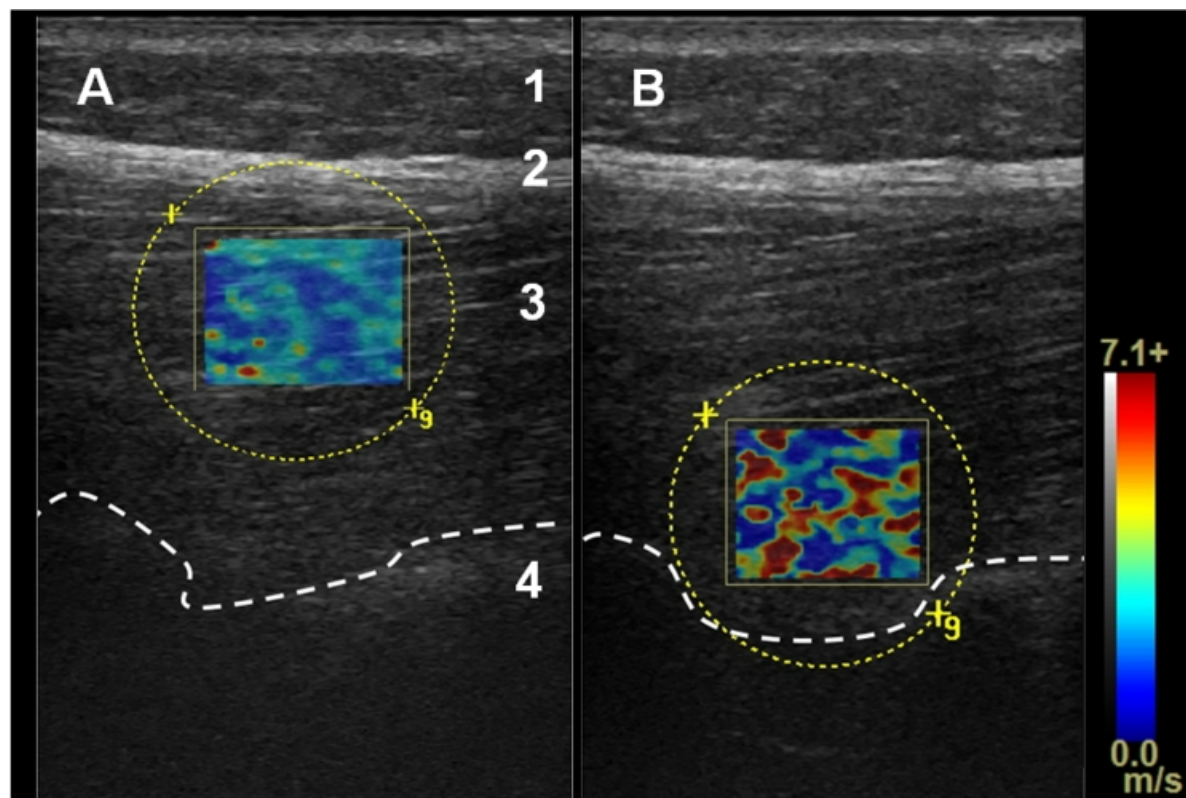


Figure 1

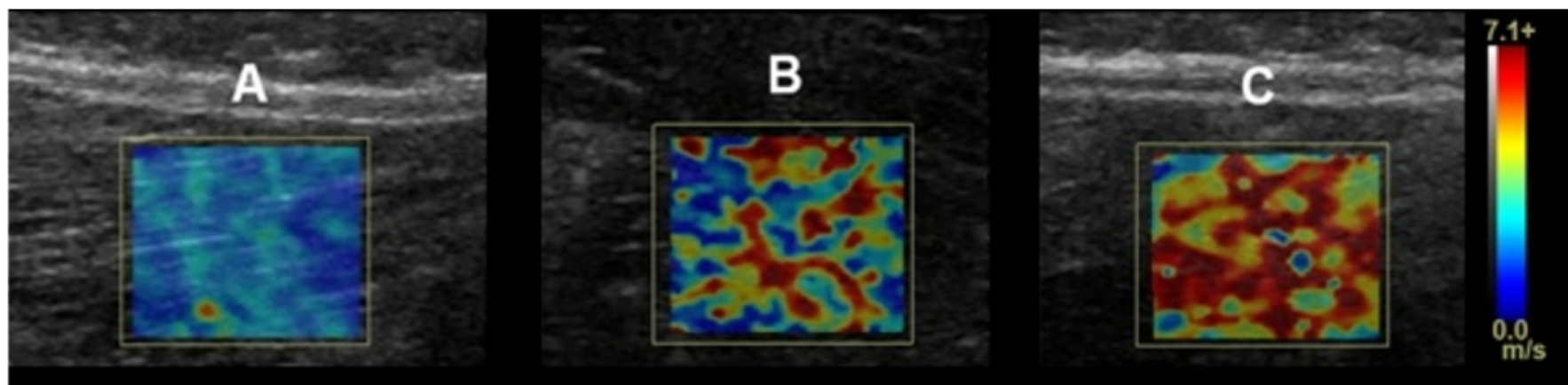


Figure 2

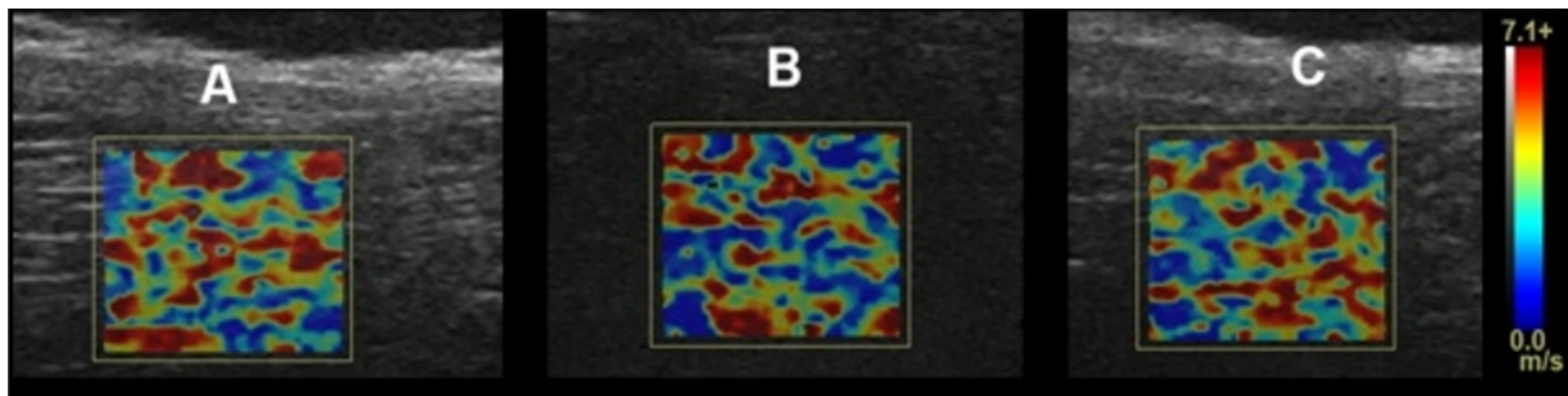
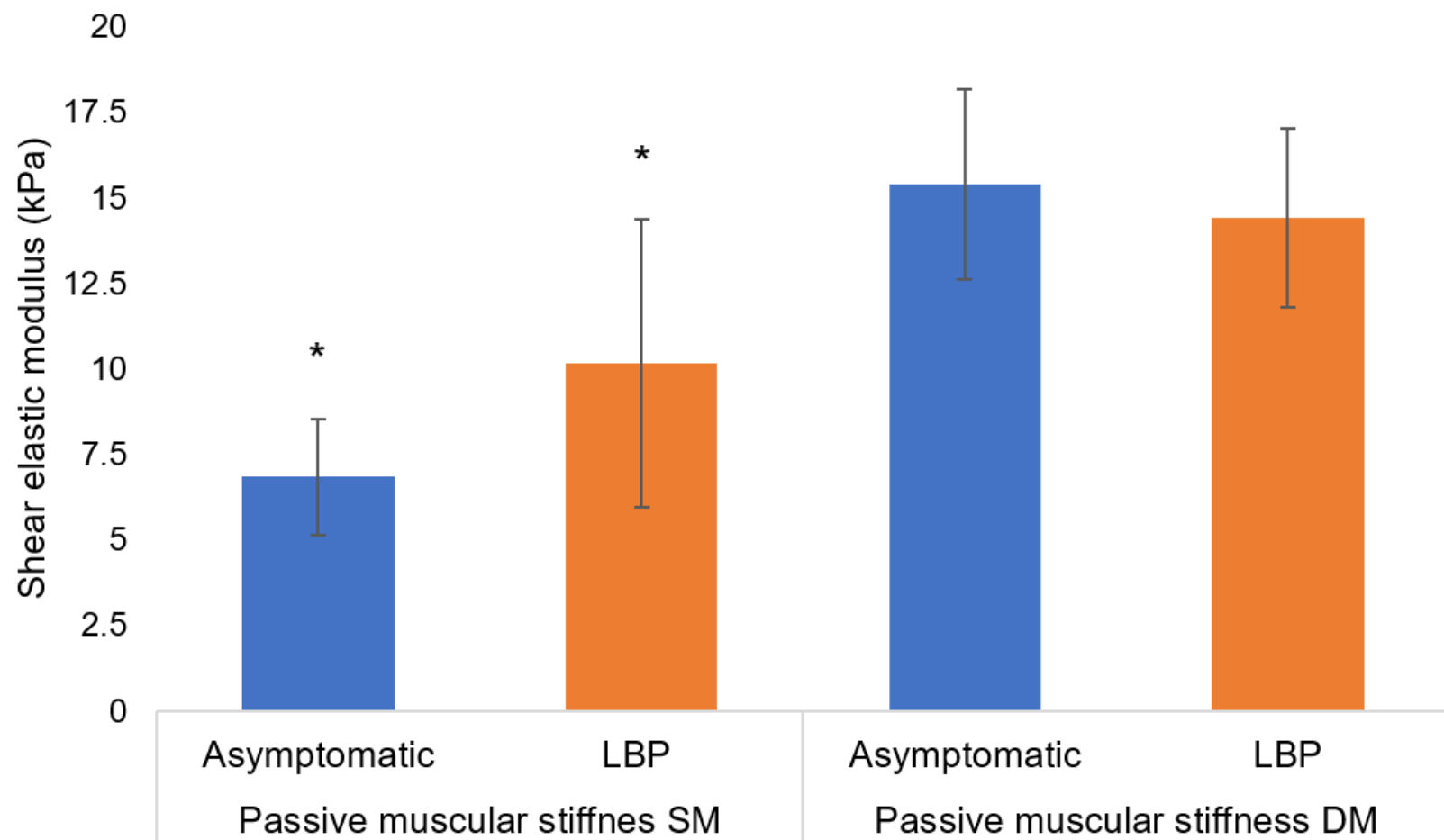
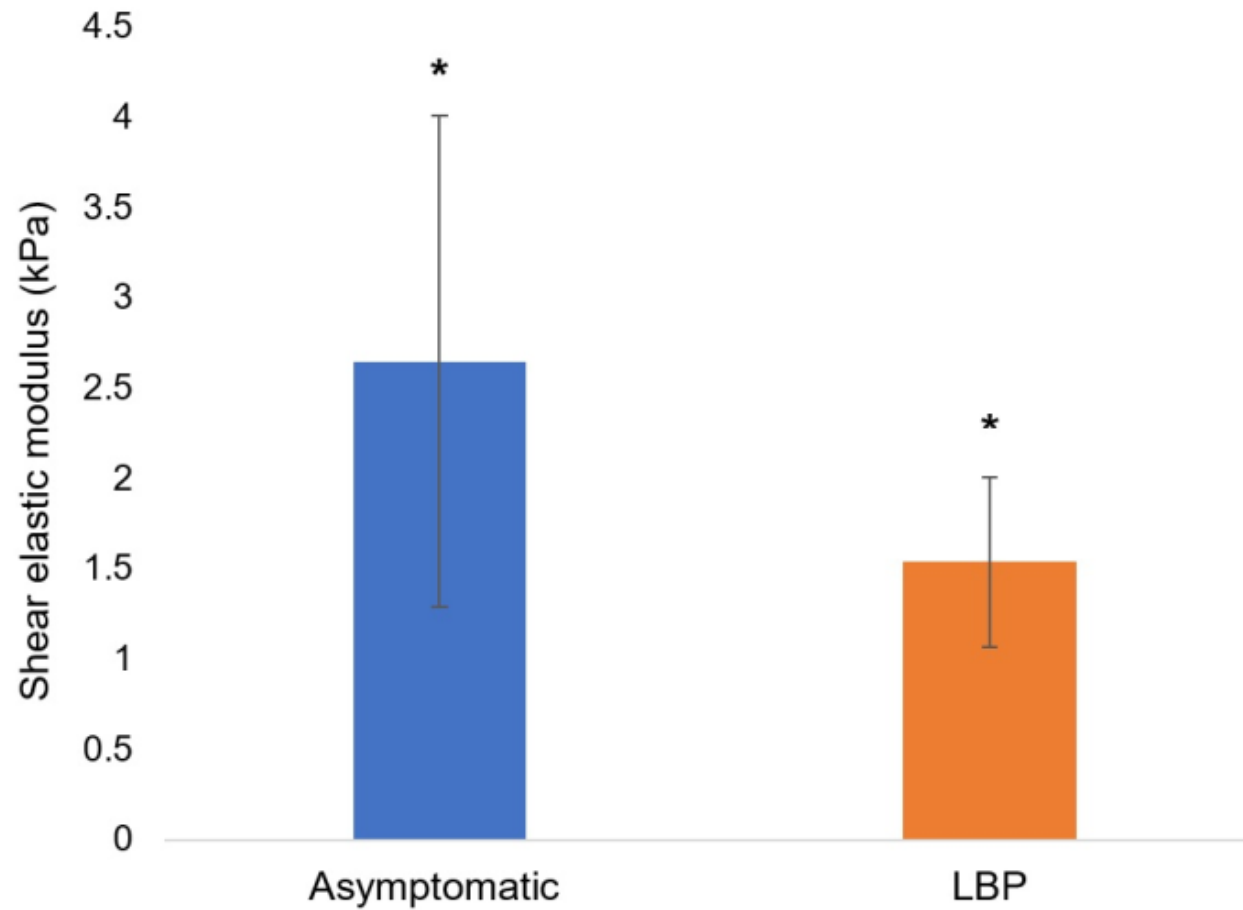


Figure 3



**Figure 4**



**Figure 5**

## Tables

**Table 1.** Baseline characteristics of the participants, measured prior to the start of data collection.

Characteristic	LBP Mean $\pm$ SD	Asymptomatic Mean $\pm$ SD	P value
Age (years)	29.4 $\pm$ 10.80	26.71 $\pm$ 5.40	$p=0.90$
Gender (% male)	46.70	53.30	$p=0.715$
BMI (Kg/m <sup>2</sup> )	25.29 $\pm$ 3.18	24.01 $\pm$ 3.42	$p=0.54$
NRS current pain (0-10)	2.27 $\pm$ 1.62		
NRS usual pain (0-10)	2.93 $\pm$ 1.98		
ODI (%)	12.7 $\pm$ 6.35		
TSK	34.4 $\pm$ 3.13		

LBP –Low Back Pain, BMI – Body Mass Index, NRS – Numeric Rating Scale for pain, ODI – Oswestry Disability Index, TSK – Tampa Scale for Kinesiophobia

**Table 2.** Values for muscular stiffness measurements and comparison of passive muscular stiffness of Superficial Multifidus (SM) and Deep Multifidus (DM) within and between groups

	Passive muscular stiffness SM Shear elastic modulus (kPa)	Passive muscular stiffness DM Shear elastic modulus (kPa)	Post hoc comparisons between muscle fibers
LBP	10.15 $\pm$ 4.21	14.41 $\pm$ 2.62	$p<0.001^*$ , 95% CIs [1.797, 6.732]
Asymptomatic	6.84 $\pm$ 1.69	15.40 $\pm$ 2.77	$p<0.001^*$ , 95% CIs [6.797, 11.670]
Post hoc comparisons between groups	$p=0.005^*$ , 95% CIs [1,175, 5.906]	$p=0.181$ , 95% CIs [-0.728, 5.906]	

LBP, low back pain; kPa, kilopascals; CIs, confidence intervals

**Carlos Murillo** is a physiotherapist who obtained his MRes in Spinal Pain from the University of Birmingham (United Kingdom) in 2018. He conducted his MRes thesis within the Centre of Precision Rehabilitation for Spinal Pain (CPR Spine); which was focused on investigating the alteration in muscular stiffness and activity of the lumbar muscles in people with low back pain. He is currently carrying out his PhD at Ghent University (Belgium) within a FWO funded research project.

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**Alison Rushton** is Reader in Musculoskeletal Rehabilitation Sciences, at the University of Birmingham, UK. She is Deputy Director/Research Co-Lead of the Centre of Precision Rehabilitation for Spinal Pain and Programme Lead for the MRes Spinal Pain. Alison has a strong research profile, with >100 publications in high impact journals including BMJ, Spine and BJSM. Her research is driven by patients and key issues in musculoskeletal clinical practice with a focus to spinal pain and dysfunction; personalising interventions to the individual patient, and developing evidence informed decision-making frameworks to inform safe/efficacious interventions. Alison is Chair of the Standards Committee of the International Federation of Orthopaedic Manipulative Physical therapy. And sits on the Editorial Board of BMC Musculoskeletal, Plos One and Musculoskeletal Science and Practice journals. Alison's contribution to musculoskeletal rehabilitation research has been recognised through the award of fellowships from the UK Chartered Society of Physiotherapy and the UK Musculoskeletal Association of Chartered Physiotherapists.

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We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property. We further confirm that any aspect of the work covered in this manuscript that has involved human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

Yours sincerely on behalf of the named authors



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I certify that all authors have seen and approved the final version of the manuscript being submitted. They warrant that the article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere.

Yours sincerely

A handwritten signature in dark ink, reading "Nicola Heneghan". The signature is written in a cursive, flowing style.

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